EasySep™ Direct HLA Crossmatch T Cell Isolation Kit

For processing 1 x 10^9 cells

Catalog #19671
#19671RF RoboSep™

Negative Selection
Document #10000005421 | Version 03

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Description

Isolate highly purified T cells directly from human whole blood, buffy coat, spleen, or lymph nodes by immunomagnetic negative selection.

The benefits of this kit include:

- 99.9% RBC depletion without the need for density gradient centrifugation, sedimentation, or lysis
- Up to 99.9% purity of isolated cells
- Fast, easy-to-use, and column-free
- Isolated cells are untouched

This kit targets non-T cells for removal with antibodies recognizing specific cell surface markers. Unwanted cells are labeled with antibodies and EasySep™ Direct RapidSpheres™ and separated using an EasySep™ magnet. Desired cells are simply collected into a new tube and are immediately available for downstream applications, such as flow cytometry crossmatch.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Direct HLA Crossmatch T Cell Isolation Cocktail	19671C	2 x 2.5 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS.
EasySep™ Direct RapidSpheres™ 50300	50300	4 x 2.5 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A suspension of magnetic particles and monoclonal antibodies in PBS.

PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) and should be refrigerated upon receipt.

Precipitate may be observed in the cocktail vial but will not affect performance.

Sample Preparation

PERIPHERAL BLOOD

For optimal RBC depletion, collect blood using heparin or acid citrate dextrose (ACD) as an anticoagulant.

For best recovery, use unprocessed human whole blood. Recovery of the desired enriched cells decreases with samples that are older than 24 hours.

The volume of sample that can be processed depends on the EasySep™ magnet used for the enrichment procedure. Samples must be placed in the required tube to properly fit into the appropriate EasySep™ magnet (see Tables 1 - 4).

BUFFY COAT

1. Add an equal volume of recommended medium to whole blood.
2. Centrifuge at 800 x g for 10 minutes at room temperature (15 - 25°C) with the brake off.
3. Remove the concentrated leukocyte band (this is the buffy coat), plus a small portion of the plasma and concentrated red blood cells (RBCs). The target is to concentrate the leukocytes approximately 5-fold while maintaining the same hematocrit (e.g. collect 2 mL of buffy coat when starting with 10 mL of whole blood).
4. Transfer buffy coat to the required tube (see Tables 1, 2, and 5).

SPLEEN or LYMPH NODE

Disrupt spleen or lymph node in PBS or Hanks' Balanced Salt Solution (HBSS) containing 2% fetal bovine serum (FBS). Remove aggregates and debris by passing the cell suspension through a pre-wetted 100 µm mesh nylon strainer. Rinse the strainer with PBS or HBSS containing 2% FBS. Centrifuge at 300 x g for 10 minutes and resuspend at 1 - 100 x 10^6 cells/mL in recommended medium.

Recommended Medium

D-PBS (Without Ca++ and Mg++; Catalog #37350).

Directions for Use – Manual EasySep™ Protocols

See page 1 for Sample Preparation and Recommended Medium. Refer to Tables 1 - 4 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Direct HLA Crossmatch T Cell Isolation Kit Protocol for WHOLE BLOOD or BUFFY COAT

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	Collect sample within the volume range.	0.5 - 1.5 mL	1 - 7 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
3	Add Isolation Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample
4	Add RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
5	Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 2.5 mL	• Top up to double the volume for samples ≤ 5 mL • Top up to 10 mL for samples > 5 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
6	Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring the enriched cell suspension* into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube
7	Add RapidSpheres™ to the new tube containing the enriched cells.	Use same volume as in step 4	Use same volume as in step 4
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
8	Remove the tube from the magnet; place the tube from step 7 (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes	RT for 5 minutes
9	Pick up the magnet, and in one continuous motion invert the magnet and tube,** pouring the enriched cell suspension into a new tube.	Isolated cells are ready for use	Use a new 14 mL tube
10	Remove the tube from the magnet; place the new tube from step 9 (without lid) into the magnet and incubate for a third separation.	---	RT for 5 minutes
11	Pick up the magnet, and in one continuous motion invert the magnet and tube,** pouring the enriched cell suspension into a new tube.	---	Isolated cells are ready for use

RT - room temperature (15 - 25°C)

* Following the first magnetic separation, the collected cells may contain a significant amount of RBCs and may look similar to the original unprocessed human whole blood sample.

** To minimize RBC contamination in the isolated cells, pour off the sample along a clean area of the tube (i.e. the opposite side to where the sample was poured in).

Table 2. EasySep™ Direct HLA Crossmatch T Cell Isolation Kit Protocol for WHOLE BLOOD or BUFFY COAT

		EASYSEP™ MAGNETS		
STEP	INSTRUCTIONS	EasyEights™ (Catalog #18103)		Easy 50 (Catalog #18002)
		5 mL tube	14 mL tube	
1	Collect sample within the volume range.	0.5 - 1.5 mL	1.5 - 7 mL	7 - 30 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)	50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)
2	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
3	Add Isolation Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
4	Add RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
5	Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 2.5 mL	• Top up to double the volume for samples ≤ 5 mL • Top up to 10 mL for samples > 5 mL	• Top up to double the volume for samples ≤ 25 mL • Top up to 50 mL for samples > 25 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes
6	Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: Collect the entire clear fraction from top to bottom. For optimal recovery, also collect a small volume of RBCs (up to 10% of the starting sample volume).	Use a new 5 mL tube	Use a new 14 mL tube	Use a new 50 mL tube
7	Add RapidSpheres™ to the new tube containing the enriched cells.	Use same volume as in step 4	Use same volume as in step 4	Use same volume as in step 4
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
8	Remove the tube from the magnet; place the tube from step 7 (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
9	Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: Collect only the clear fraction.	Use a new 5 mL tube	Use a new 14 mL tube	Use a new 50 mL tube
10	Remove the tube from the magnet; place the new tube from step 9 (without lid) containing the enriched cells into the magnet and incubate for a third separation.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
11	Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: Collect only the clear fraction.	Isolated cells are ready for use	Isolated cells are ready for use	Isolated cells are ready for use

RT - room temperature (15 - 25°C)

*** Collect the entire supernatant, all at once, into a single pipette (e.g. for EasyEights™ 5 mL tube, use a 2 mL serological pipette [Catalog #38002]; for EasyEights™ 14 mL tube, use a 10 mL serological pipette [Catalog #38004]).

Table 3. EasySep™ Direct HLA Crossmatch T Cell Isolation Kit Protocol for SPLEEN or LYMPH NODE

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	Collect sample within the volume range.	0.5 - 1.5 mL	1 - 7 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add Isolation Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add RapidSpheres™ to sample. Mix by gently pipetting up and down 2 - 3 times.	50 µL/mL of sample	50 µL/mL of sample
5	Add recommended medium to top up the sample to the indicated volume.	Top up to 2.5 mL	• Top up to double the volume for samples ≤ 5 mL • Top up to 10 mL for samples > 5 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 3 minutes
6	Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring the enriched cell suspension into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube
7	Add RapidSpheres™ to the new tube containing the enriched cells. Mix by gently pipetting up and down 2 - 3 times.	Use same volume as in step 4	Use same volume as in step 4
8	Remove the tube from the magnet; place the tube from step 7 (without lid) into the magnet and incubate for a second separation.	RT for 3 minutes	RT for 3 minutes
9	Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring the enriched cell suspension into a new tube.	Isolated cells are ready for use	Isolated cells are ready for use

RT - room temperature (15 - 25°C)

Table 4. EasySep™ Direct HLA Crossmatch T Cell Isolation Kit Protocol for SPLEEN or LYMPH NODE

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	EasyEights™ (Catalog #18103)	
		5 mL tube	**14 mL tube**
1	**Collect sample within the volume range.**	0.5 - 1.5 mL	1 - 7 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	**Add Isolation Cocktail to sample.** **NOTE: Do not vortex cocktail.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
3	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
4	**Add RapidSpheres™ to sample. Mix by gently pipetting up and down 2 - 3 times.**	50 µL/mL of sample	50 µL/mL of sample
5	**Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.**	Top up to 2.5 mL	• Top up to double the volume for samples ≤ 5 mL • Top up to 10 mL for samples > 5 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 3 minutes
6	**Carefully pipette*** (do not pour) the enriched cell suspension into a new tube.** **NOTE: Collect the entire clear fraction from top to bottom. For optimal recovery, also collect a small volume of RBCs, if present (up to 10% of the starting sample volume).**	Use a new 5 mL tube	Use a new 14 mL tube
7	**Add RapidSpheres™ to the new tube containing the enriched cells. Mix by gently pipetting up and down 2 - 3 times.**	Use same volume as in step 4	Use same volume as in step 4
8	**Remove the tube from the magnet; place the tube from step 7 (without lid) into the magnet and incubate for a second separation.**	RT for 3 minutes	RT for 3 minutes
9	**Carefully pipette*** (do not pour) the enriched cell suspension into a new tube.** **NOTE: Collect only the clear fraction.**	Use a new 5 mL tube **For lymph node**: Isolated cells are ready for use	Use a new 14 mL tube **For lymph node**: Isolated cells are ready for use
10	**Remove the tube from the magnet; place the new tube from step 9 (without lid) containing the enriched cells into the magnet and incubate for a third separation.**	**For spleen**: RT for 3 minutes	**For spleen**: RT for 3 minutes
11	**Carefully pipette*** (do not pour) the enriched cell suspension into a new tube.** **NOTE: Collect only the clear fraction.**	**For spleen**: Isolated cells are ready for use	**For spleen**: Isolated cells are ready for use

RT - room temperature (15 - 25°C)

*** Collect the entire supernatant, all at once, into a single pipette (for EasyEights™ 5 mL tube, use a 2 mL serological pipette [Catalog #38002]; for EasyEights™ 14 mL tube, use a 10 mL serological pipette [Catalog #38004]).

Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 5 for detailed instructions regarding the RoboSep™ procedure.
NOTE: If using RoboSep™-S, ensure the software is at least v.1.2.0.2 and RoboSep™ Direct-compatible carousel is installed. Contact us at techsupport@stemcell.com for more information.

Table 5. RoboSep™ Direct HLA Crossmatch T Cell Isolation Kit Protocol for WHOLE BLOOD, SPLEEN, LYMPH NODE, or BUFFY COAT

STEP	INSTRUCTIONS	RoboSep™ (Catalog #21000) 
1	**Prepare sample at the indicated cell concentration within the volume range.**	**For spleen or lymph node:** 1 - 6 mL at 1 - 100 x 10^6 cells/mL **For blood:** 1 - 6 mL **For buffy coat:** 2 - 5 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	**Select protocol.**	• EasySep Direct HLA Crossmatch T Cell Isolation 19671 - For WB, Spleen, LN • EasySep Direct HLA Crossmatch T Cell Isolation 19671 - For WB, Spleen, LN - High RBC Depletion† • EasySep Direct HLA Crossmatch T Cell Isolation 19671 - For BC§
3	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds
4	**Load the carousel.**	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
5	**Unload the carousel when the run is complete.**	Isolated cells are ready for use

† This protocol provides high red blood cell depletion, with a slight reduction in recovery
§ This protocol uses two times the EasySep™ reagents

Notes and Tips

REMOVAL OF RESIDUAL RBCs IN THE ISOLATED CELLS

Typically, further RBC depletion is not required following cell isolation. If residual RBCs are visible in the isolated cell pellet following centrifugation after the end of the protocol, resuspend in a small volume (0.2 - 2.5 mL) of recommended medium or desired culture medium and place in a smaller EasySep™ magnet for an additional 5-minute separation. Collect the supernatant; the isolated cells are ready for use in downstream applications. Residual RBCs may also be lysed using Ammonium Chloride Solution (Catalog #07800).

ASSESSING PURITY

For purity assessment of T cells by flow cytometry, use the following fluorochrome-conjugated antibody clones:

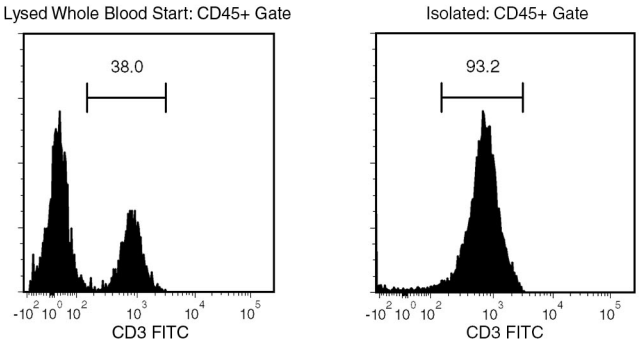
- Anti-Human CD3 Antibody, Clone SK7 (Catalog #60127), or
- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011), and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018)

NOTE: It is recommended to assess purity on CD45+ cells to exclude debris, platelets, and RBCs. Include a viability dye if necessary (e.g. Propidium Iodide [Catalog #75002]; 7-AAD [7- Aminoactinomycin D; Catalog #75001]).

NOTE: User is responsible for validating the performance of the isolated cells in downstream assays.

Data

Starting with human whole blood from normal healthy donors, the T cell (CD3+) content of the non-lysed final isolated fraction typically ranges from 89 - 99.9% (gated on CD45).



In the above example, the T cell (CD3+) content of the lysed whole blood start sample and non-lysed final isolated fraction is 38.0% and 93.2% (gated on CD45), respectively.